SEXUAL PHASES IN THE AMERICAN OYSTER (OSTREA VIRGINICA)

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During the past three years there has been an accumulation of evidence which indicates that in at least three of the so-called dioecious species of the genus *Ostrea* a change of sex frequently occurs from season to season or between early life and full maturity. It may be recalled that at least ten of the more than sixty described species of the genus are regularly hermaphroditic and larviparous. Some of these exhibit a rhythmical sequence of alternating male and female phases, as Spärck (1925) and Orton (1926–27) have so fully described for *O. edulis* and as Coe (1931, 1932) has more recently shown for *O. lurida*.

Moreover, sex determination in other bivalves, as well as in some gasteropod mollusks, has long been known to be in such a labile condition that environmental changes may profoundly alter its expression. It may not be surprising, therefore, to find that changes of sex, especially protandry, as well as various aspects of intersexuality, have been found to occur in dioecious and oviparous species of oysters. For example, Roughley (1928) concluded from his observations on *O. cucullata* that that species, formerly considered dioecious, is regularly protandric, for nearly all the very small individuals were found to be males. An experiment by Amemiya (1929) has been thought to indicate that in *O. gigas* the sexual phase of each individual is determined each winter without influence from its previous sexual condition.

Many years ago Stafford (1913) found indications that the American oyster (*O. virginica*) is protandric on the Canadian coasts and presented evidence that the young animal becomes a sexually mature male when it has reached a length of about 25 mm. At 32 mm. the gonads may be distended with spermatozoa.

More recently Burkenroad (1931) has also shown that protandry occurs in this species on the coast of Louisiana. He concluded that although the sexes of the adults are morphologically separate, each individual is essentially a protandrous hermaphrodite. His evidence indicates that close association with large oysters causes some individuals to assume or retain the male phase, although other oysters of the same size, but growing singly, are predominantly females. He found small individuals to be almost always males regardless of their associations and interpreted his evidence as indicating that the likelihood of large oysters being males decreases rapidly as the distance from other individuals increases.

Further evidence of protandry in this species has been furnished by Needler (1932) from oysters collected at various localities on the North Atlantic coasts of the United States and Canada. She con-

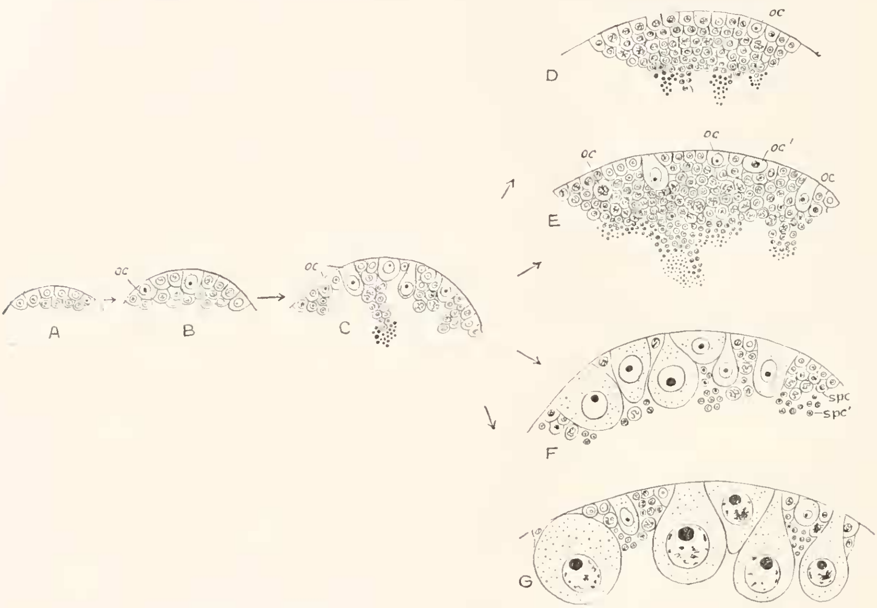

FIG. 1. Diagrams showing the primitive intersexual gonad and its transformation into the definitive spermary and ovary. *A*, early gonad with undifferentiated cells; *B*, intersexual phase, with differentiating ovocytes (*oc*); *C*, later intersexual phase with preliminary abortive spermatogenesis; *D*, spermary with only a few small ovocytes; *E*, spermary with many ovocytes, some of which (*oc'*) are in process of degeneration; *F*, young ovary, with many spermatocytes (*spc*), some of which (*spc'*) are pycnotic; *G*, nearly ripe ovary with residual cells and degenerating spermatocytes.

cludes that the majority of individuals first mature as males and that many of them later change to females. The change may be hastened by favorable nutritive conditions and may possibly be retarded by close association with older females. She observed one instance where a three-year-old male changed into a functional female in the interval preceding the next breeding season.

In order to determine more precisely the sequence of these changes in sexuality and the histological activities which accompany them, the gonads of a large number of oysters have been examined at frequent intervals during the first two years of their lives. Some of this material was collected from rocks along the shore of Long Island Sound in the vicinity of New Haven; some was taken from various natural oyster beds near Woods Hole, Massachusetts; many samples both of cultivated and untransplanted oysters from Quinnipiac River, New Haven Harbor, and Long Island Sound have been supplied by Mr.

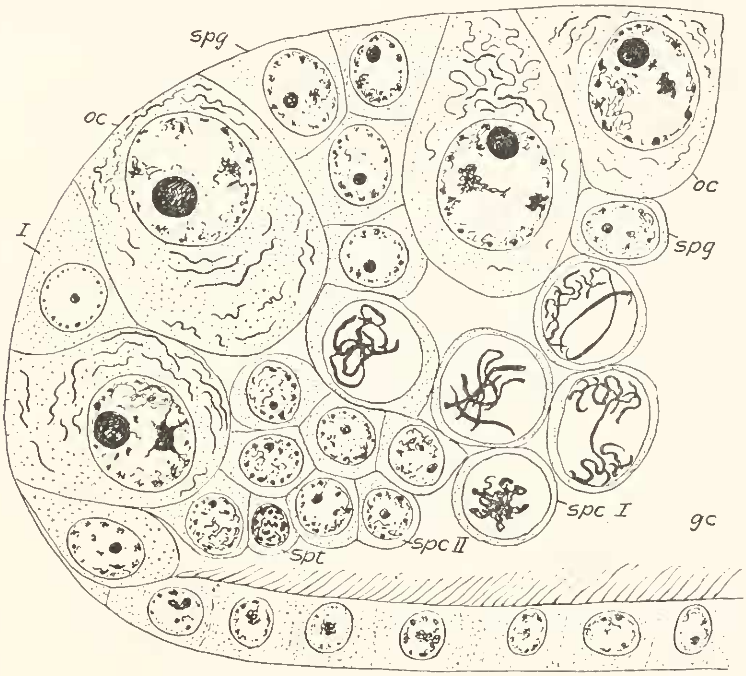

FIG. 2. Primary gonad in animal about four months of age, showing large ovocytes (*oc*), spermatogonia (*spg*), and primary spermatocytes (*spc* I) in spireme phase, with secondary spermatocytes (*spc* II) and a single spermatid (*spt*) bordering the ciliated genital canal (*gc*).

Howard W. Beach, Chairman of the Research Committee of the Oyster Growers and Dealers Association of North America, while a most instructive series of first-year stages was furnished by Mr. J. B. Glancy from the floats of the same Association at West Sayville, Great South Bay, Long Island.

From these collections the early development of the gonads and their transformations in the course of successive phases of sexuality have been studied both in life and by means of serial sections.

Development of the Primary Bisexual Gonads

The profusely branching tubules of the primitive gonads can be found in young oysters at the age of six to eight weeks after setting or when the shell has reached a length of 6–10 mm. They ramify within the thin layer of connective tissue immediately beneath the body walls as Stafford (1913) has previously described.

In these primary gonads the germinal epithelium consists of a thin layer of morphologically undifferentiated cells which lie upon the inner side of the tubular gonad (Fig. 1), while the cells which line the outer

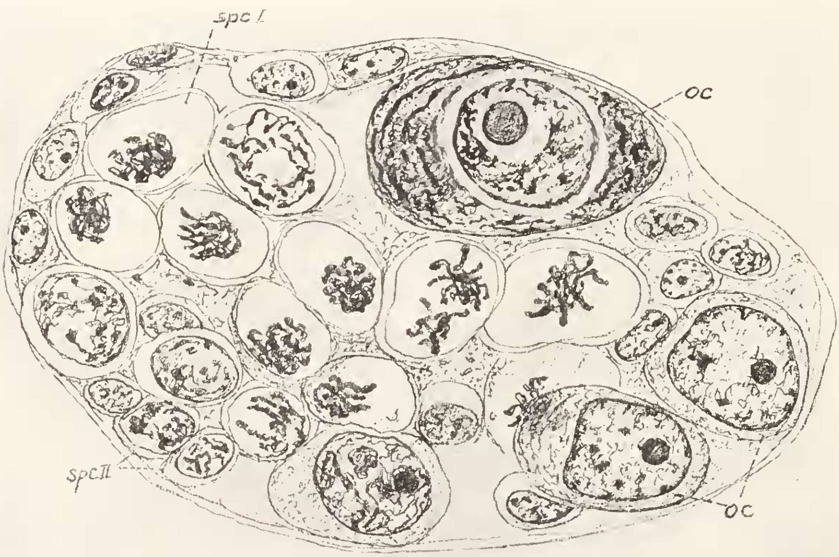

Fig. 3. Primary intersexual gonad, showing primary spermatocytes (*spc* I) and ovocytes in synapsis, secondary spermatocytes (*spc* II) and several large ovocytes (*oc*). Age about four months.

wall, adjacent to the epidermis, become differentiated into the ciliated epithelium of the genital canals (Figs. 2, 4, 5, 6) as Hoek (1883–84) so fully described many years ago for *O. edulis* and as Coe (1932) verified in *O. lurida*.

A few weeks later the germinal epithelium shows a differentiation into larger and smaller cells. The former are soon recognizable as ovocytes by the presence of fibrillar mitochondrial bodies, while many of the latter show by their rapid proliferation and later specialization that they belong to the male germ line.

By the middle of October, at the age of about three months, the shells of some individuals have become 20–25 mm. in length, and in these the gonads have already become distinctly bisexual (Figs. 2–6). A typical section through the genital canal at this age (Fig. 2) shows more or less numerous indifferent cells remaining along the inner border, interspersed with large ovocytes in which the coarse mitochondrial filaments of the so-called yolk nuclei are always con-

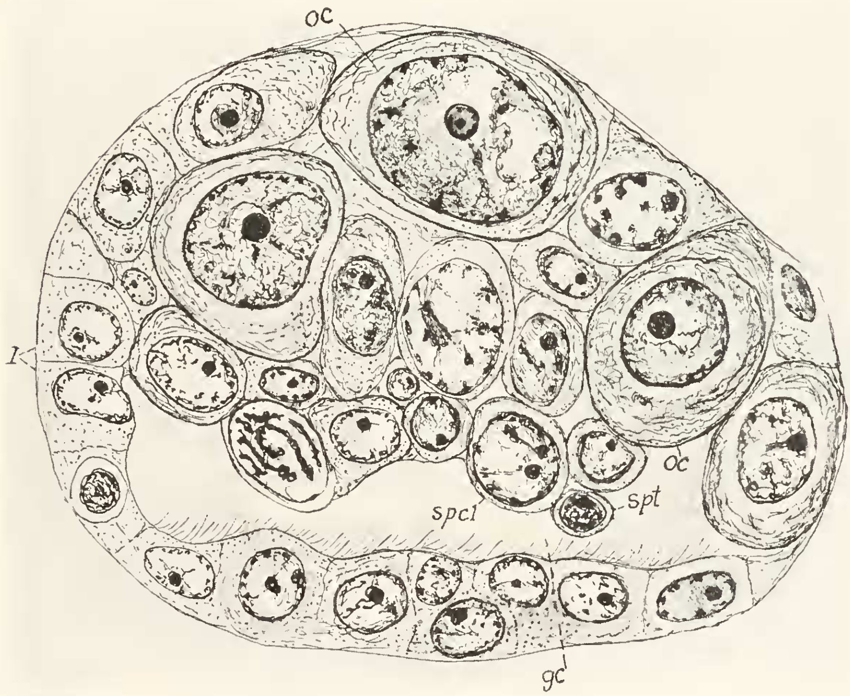

FIG. 4. Primary intersexual gonad in an animal about four months of age, showing indifferent cells (I) and several large ovocytes (*oc*), with spermatocytes (*spc* I) and a single spermatid (*spt*) bordering the ciliated genital canal (*gc*).

spicuous. Morphologically less well differentiated are the ovogonia and spermatogonia which resemble each other so closely that they cannot ordinarily be distinguished. Both types of cells then pass through similar synaptic phases accompanied by spiremes of coarse, densely-staining chromosomes (Figs. 2, 3, 9*B*, 9*C*) as they transform into ovocytes and primary spermatocytes respectively.

The spermatogonia proliferate rapidly, leading to the formation of the numerous spermatocytes which soon give the intersexual gonad a predominantly male appearance. In some individuals the spermatocytes complete their meiotic divisions to form spermatids, thereby accentuating the resemblance of the gonad to a spermary (Figs. 4–6).

In other animals of the same age the gonads may retain a closer similarity to an ovary (Figs. 2, 3), and it is improbable that any spermatids are formed in every individual. All degrees of inter-

FIG. 5. Primary gonad, with ovocytes (*oc*) and primary spermatocytes (*spc* I) in synapsis; a few spermatids (*spt*) are already present on the border of the genital canal (*gc*); age about four months.

sexuality are found, although the vast majority of individuals are predominantly male. Not infrequently some parts of the system may assume a distinctly male appearance while adjacent follicles are characteristically female, as shown in Fig. 6.

The process of spermatogenesis does not continue through the winter, however, in the localities investigated and no functional spermatozoa are formed. The gonads may continue to increase somewhat in size during December, with an increase in the numbers of secondary spermatocytes and spermatids. Their activities are then interrupted until there is a rise in the temperature of the water the following spring.

Hibernation

During the long period of hibernation, when the valves of the oyster remain closed, relatively minor changes occur in the primary gonads and these are mainly regressive in character. There is little, if any, increase in the size of the gonads during the oyster's first

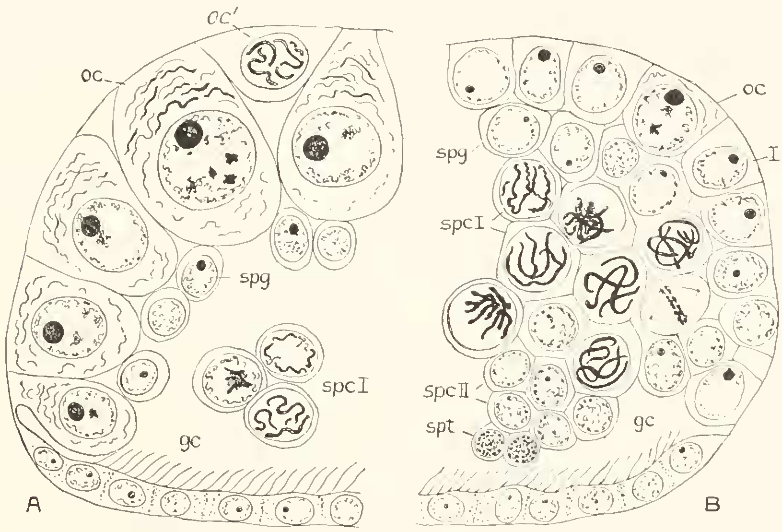

Fig. 6. Portions of two follicles from the same individual at the age of about four months: one of these (*A*) is predominantly of the female type while the other (*B*) consists mainly of spermatogenic cells, although both show some indications of their intersexual character; *oc′*, young ovocyte in spireme phase; other letters as in Fig. 2.

winter or in the numbers of their constituent cells. On the contrary, many of the previously differentiated cells become obviously abnormal and evidences of cytolysis are frequently seen. Remains of disintegrated cells sometimes occur in the lumens of the follicles and ducts.

Transformation to Functional Gonads

At West Sayville the animal retains its primary or immature gonad throughout the winter as the figures in Table I will show. As the water becomes warmer, however, spermatogenesis is resumed in some of the larger animals and in March about one-fourth of the young oysters examined had transformed into males. No females were recognized until a month later.

In an occasional individual, due apparently to poor nutrition, the primary bisexual type of gonad is retained until the second year, at which time a few functional spermatozoa may be formed.

Males

The transformation of the bisexual gonad into a spermary consists in the proliferation of spermatogonia and spermatocytes, usually accompanied by the disintegration of some of the previously formed ovocytes (Fig. 1). There is a rapid extension of the branching system of follicles, the new branches being, as a rule, exclusively male in appearance, for the reason that the ovocytes usually found in the older

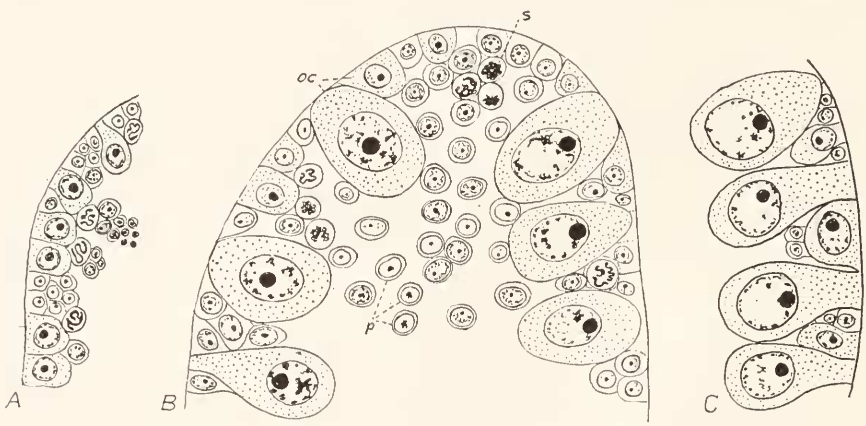

FIG. 7. Transition stages in the development of the mature ovary (*C*) from the primitive intersexual gonad (*A*). *B*, developing ovary in an individual about ten months of age, showing numerous small pycnotic cells (*p*) in the lumen and between the ovocytes (*oc*); *s*, cells in spireme phase.

parts of the system are not carried into the new follicles (Fig. 8). But this rule is not without exceptions, for many grades of intersexuality occur and the gonad is classed as a spermary in this report if it has a distinct preponderance of spermatogenic cells, even though many ovocytes are situated along the walls of the follicles (Fig. 12, *A*). The term hermaphrodite is reserved for those cases in which there are extensive areas or large masses of the cells representing each of the sexes (Fig. 12, *B*).

Females

As the season advances an increasing proportion of the young oysters attain sexual maturity, as shown in Table I. During April

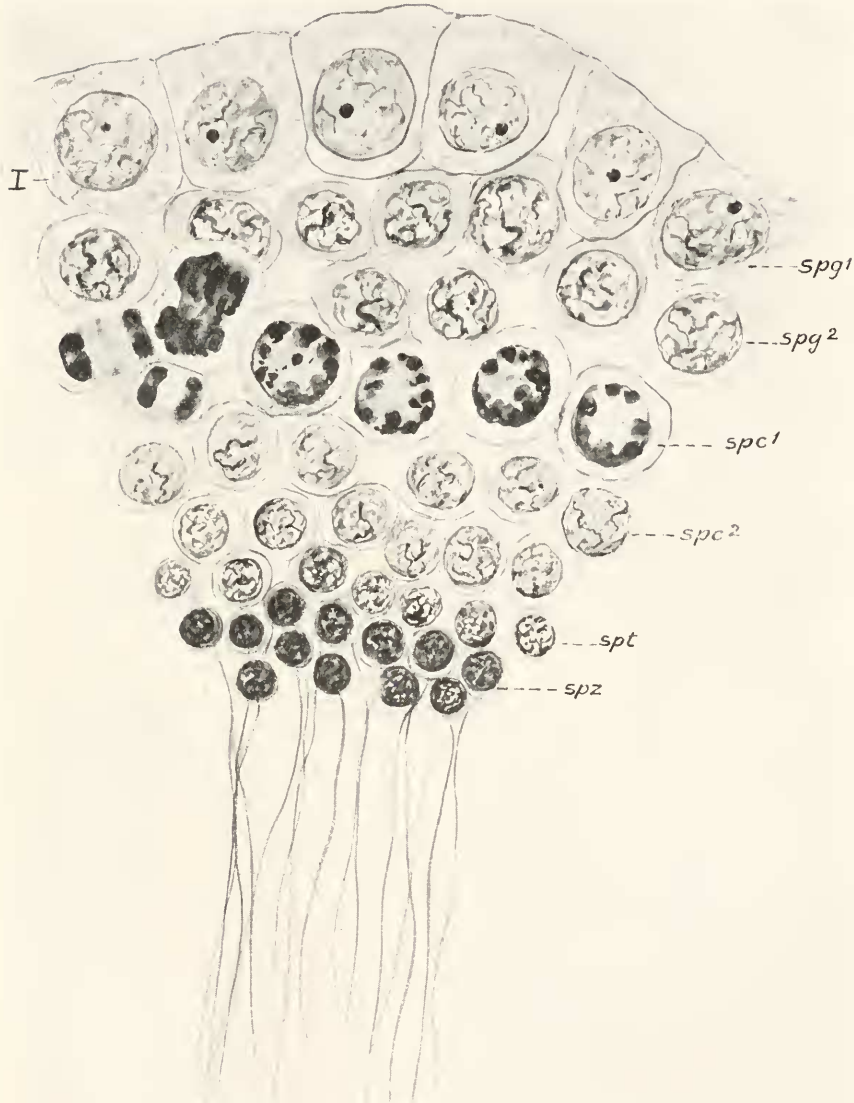

FIG. 8. Mature spermary, showing indifferent residual cells (*I*) and normal spermatogenesis; *spg*[1], *spg*[2], primary and secondary spermatogonia; *spc*[1], *spc*[2], primary and secondary spermatocytes; *spt*, spermatids; *spz*, mature spermatozoa.

the first females were found and these showed the transition of the intersexual gland into an ovary (Fig. 7). This process is accomplished by the concurrent growth of the primitive ovocytes, with additions from the small ovogonia, and the pycnosis and eventual cytolysis of such secondary spermatocytes and spermatids as may be present (Fig. 7).

Many indifferent cells and presumably some of the spermatogonia remain as residual cells even after ovulation has occurred. The first crop of eggs discharged is very small, as only a minute proportion of the definitive ovocytes reach maturity until after the first ovulation

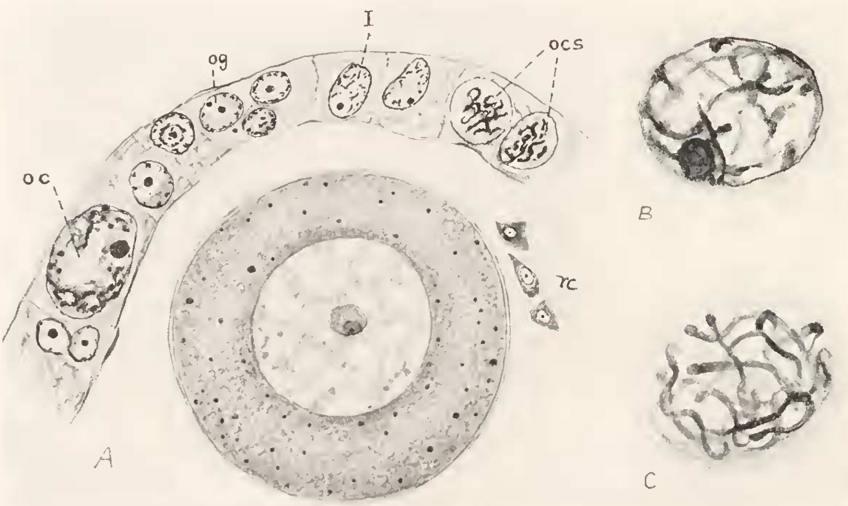

FIG. 9. *A*, mature ovary, showing a single ripe ovum and the residual cells of the follicle; *I*, indifferent residual cells; *og*, ovogonia; *ocs*, young ovocytes in synaptic phase; *oc*, residual ovocyte; several pycnotic residual cells (*rc*) are free in the lumen. *B*, *C*, spireme stages in young ovocytes.

(Fig. 9). The genesis of the ripe ovum, measuring about .05 mm. in diameter has been so fully described that further discussion here is unnecessary. The spawning reactions of both male and female have been fully investigated in this and other species by Prytherch (1924) and by Galtsoff (1932).

Functional Hermaphrodites

True hermaphroditism in the adult American oyster had been considered a rarity until Burkenroad (1931) reported that about 1 per cent of the general population on the coast of Louisiana belonged in this category. Needler (1932) also found about the same percentage of hermaphrodites from one locality and a smaller proportion from other areas on the coast of Prince Edward Island. Those that she found were in their third year except one each in their fourth and fifth years.

Although the primary gonad is normally bisexual and at least some degree of intersexuality is usually found in sexually mature yearlings, only 4 among 96 such individuals from W. Sayville and 4 among 389 from New Haven Harbor were classed as true hermaphrodites. These showed approximately equal areas of male and female cells (Fig. 12, *B*). Several others were considered to be hermaphroditic males, being provided with spermaries of normal appearance except for the presence of scattered ovocytes (Fig. 12, *A*), sometimes fully grown.

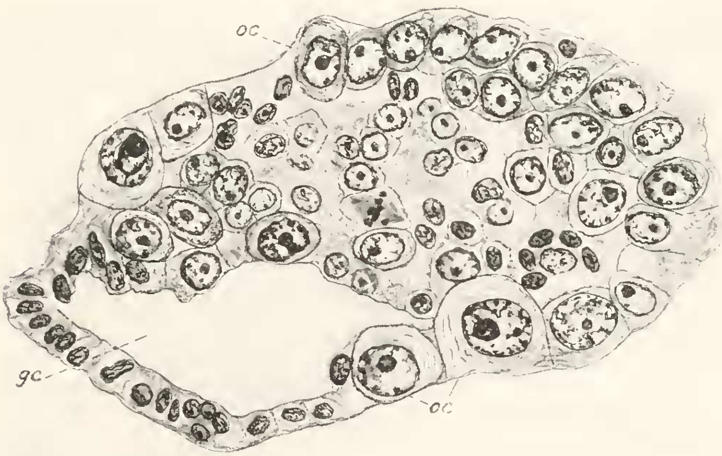

FIG. 10. Spermary early in second year, showing its continued intersexual character; *gc*, genital canal; *oc*, ovocytes.

SELF-FERTILIZATION

Among 55 sexually mature yearlings taken from the culture floats at West Sayville on June 23 were two functional hermaphrodites. Many of the ova in each of these proved capable of self-fertilization and apparently normal development in spite of the vast excess of sperm present. Needler (1932) has reported a similar observation.

SPERMATOGENESIS

The successive stages in the process of spermatogenesis are similar to those described by Coe (1932) for *O. lurida* except that the spermato-

FIG. 11. Spermary at end of second year, showing its continued intersexuality by the presence of large ovocytes (*oc*) on wall of follicle; letters as in Fig. 2.

cytes and spermatids are free to separate immediately after their formation (Fig. 8), instead of adhering in masses as they do in the latter species where the spermatozoa are united into sperm-balls.

Gonads at the End of the Breeding Season and During the Second Year

Such individuals as become sexually mature during their first year produce but a relatively small number of gametes, retaining as residual cells a large proportion of the germinal cells composing the gonads. The residual germinal epithelium of the ovary is similar to that shown in Fig. 9, *A* (except for the absence of the single ripe ovum), while Fig. 10 shows a section of a spermary shortly after activity has been resumed near the beginning of the second year.

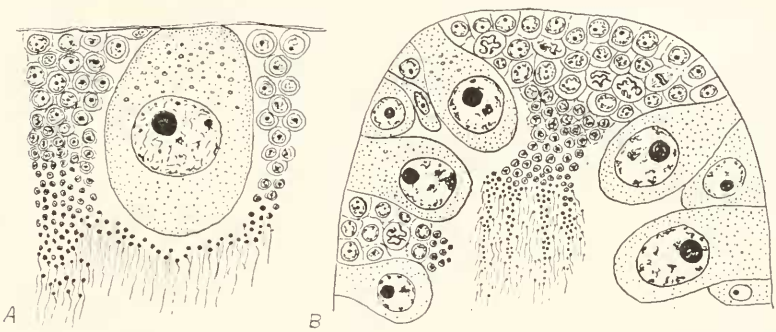

Fig. 12. *A*, partial hermaphroditism; portion of spermary of hermaphroditic male, showing one of the scattered ovocytes. *B*, complete hermaphroditism; gonad with about equal areas of ovarian and spermatogenic tissue. Age about ten months.

The gonads of both sexes after spawning usually retain indications of their continued intersexual character; in the males by the presence of ovocytes along the walls (Figs. 10, 11), and in the females by groups of small cells similar to those shown in Fig. 7. In the latter case, however, there is as yet no proof that these small cells actually belong to the male line, for active spermatogenesis has not been found in an individual classed as a female. A spermary may have few or many ovocytes which sometimes produce yolk in a normal manner, but the reverse conditions seem not to hold except in the relatively small proportion of individuals which exhibit functional hermaphroditism.

Sex Reversal

The observations of both Burkenroad (1931) and Needler (1932) indicate a strong tendency toward protandry in this species. The former concluded that the change to the female phase takes place when the shell of the young animal has reached a length of about 40 mm., while Needler has positively proved that a change of sex may occur as late as the third or fourth year.

It has hitherto been an open question whether all individuals are protandric; that is, whether the relatively few oysters which spawn as females at the end of their first year may have passed through a preliminary male phase the preceding autumn. It can now be answered that such is not the case in the localities under consideration. The ovary of yearling females develops directly out of the primary intersexual gonad by the growth of the primordial ovocytes and the elimination of spermatocytes and spermatids before spermatogenesis has been completed. The preliminary male phase is thus abortive in these localities although such may not be the case in the warmer areas farther south along the coast.

Correlation of Age, Size, and Sex During the First Year

It has been stated above that at West Sayville, Long Island, a few of the more rapidly growing young oysters become sexually mature as males during March of their first year. Females were first recognized in April and these were mainly among the largest individuals of the group.

Tables I and III show the numbers of individuals of each sex and of each size found at three different periods preceding the breeding season and once after spawning had commenced. The average size of the young females always exceeds that of the sexually mature males of the same age if considerable numbers of each are considered. This rule is shown by Needler (1932) to hold also for oysters at the age of two and of three years, but in still older animals the males are said to average as large as those of the other sex.

The greater size of the females of the younger ages may be correlated (*a*) with a more efficient metabolism associated with the female sex mechanism, or (*b*) greater activity of the female in obtaining food, or (*c*) the actual differentiation of the individual into a female as the result either of its inherent metabolic potentialities or its favorable environmental conditions, or both.

And, conversely, the responsible agency for the determination of maleness and slower growth may be a less favorable metabolism, either genetic or environmental, or, conceivably, the retarding influence of older, associated individuals of either sex.

Inspection of Table I shows clearly that the collection made on March 23 represents a large proportion of oysters that would have become sexually mature later in the season and these would presumably have included both sexes. Omitting this collection and combining the two groups taken April 29 and May 21 shows (Table II) that shortly before the beginning of the breeding season in June, 41.6 per cent of 149 specimens sent for examination were still immature, while 41 per cent were males, 1.3 per cent hermaphroditic, and 16 per cent were females. By a curious coincidence these figures agree surprisingly closely with those obtained by Needler (1932) from the same locality

Table I

Correlation of Size and Sex during First Year, W. Sayville

Length	March 23			April 29			May 21				June 23			
	Im.	M.	F.	Im.	M.	F.	Im.	M.	H.	F.	Im.	M.	H.	F.
mm.														
10–19 . .	6	—	—	16	—	—	4	—	—	—	2	—	—	—
20–29 . .	6	—	—	19	1	—	8	—	—	—	5	3	—	—
30–39 . .	7	1	—	4	12	2	9	14	—	1	2	8	—	—
40–49 . .	7	3	—	1	12	1	1	7	—	1	—	11	1	1
50–59 . .	—	2	—	—	5	10	—	7	2	4	—	7	—	7
60–69 . .	—	1	—	—	—	3	—	3	—	2	—	12	1	1
70–79 . .	—	—	—	—	—	—	—	—	—	—	—	—	—	3
Total. . .	26	7	0	40	30	16	22	31	2	8	9	41	2	12

Table II

Correlation of Size and Sex; W. Sayville. Groups from April 29, May 21, and June 23 combined.

	Total	Im.	p.c.	M.	p.c.	H.	p.c.	F.	p.c.
Less than 40 mm.	110	69	62.7	38	34.5	0	0	3	2.7
More than 40 mm.	103	2	2.0	64	62.1	4	4.0	33	32.0
Less than 50 mm.	146	71	48.6	68	46.6	1	0.9	6	4.1
More than 50 mm.	67	0	0	34	50.7	3	4.5	30	44.8
Total.	213	71	33.3	102	47.9	4	2.0	36	17.0

Im., immature; sex not determinable; M., male; H., hermaphrodite; F., female.

at the end of June, 1931. She found 45.3 per cent immature, 38.7 per cent males, and 16 per cent females among 119 individuals which she examined.

On June 23, 1932, however, the writer found that only 14 per cent of the 64 samples studied were still immature, while 64 per cent were males, 3 per cent functional hermaphrodites, and 19 per cent females

(Table I). The smaller proportion of sexually mature yearlings in 1931 as compared with 1932 may be accounted for by the somewhat higher temperature of the water during the growing season of the latter year.

Combining the three groups examined April 29, May 21, and June 23, 1932, numbering 213 individuals, shows 71, or 3.33 per cent, immature; 102, or 47.9 per cent, males; 4, or 2 per cent, functional hermaphrodites; and 36, or 17 per cent, females (Table II).

Considering only the 149 individuals of the present collections that were sexually differentiated we find that they comprise 109 males; 4 hermaphrodites, and 36 females, a percentage ratio of 73.2, 2.7, and 24.2 respectively.

TABLE III

Correlation of Size Groups and Sex First Year; W. Sayville

Length	March 23			April 29			May 21				June 23			
	Im.	M.	F.	Im.	M.	F.	Im.	M.	H.	F.	Im.	M.	H.	F.
mm.														
Less than 40	19	1	0	39	13	2	21	14	0	1	9	11	0	0
More than 40	7	6	0	1	17	14	1	17	2	7	0	30	2	12
Less than 50	26	4	0	40	25	3	22	21	0	2	9	22	1	1
More than 50	0	3	0	0	5	13	0	10	2	6	0	19	1	11
Total	33			86			63				64			

Im., immature; sex not determinable; M. male; H. hermaphrodite; F. female.

At the end of June, 1931, Needler found 46 males and 19 females among the 65 sexually mature individuals which she examined from the same locality. Combining these figures with those of Table II gives a total of 155 males, 4 hermaphrodites, and 55 females, a ratio of nearly 3 males to 1 female.

Of the sexually mature individuals less than 40 mm. in length (Table III) there were 39 males to 3 females, but those of larger size showed 70 males and 33 females. But in the class measuring more than 50 mm. in length (Table III) there are nearly as many females as males, indicating a rather definite correlation of size and sex, as Burkenroad (1931) has shown for the general population and Needler (1932) for the age groups. Probably the most reliable correlation of sex and size is shown by the collection made during the early part of the breeding season (June 23), representing as nearly as could be estimated an average sample of the entire population remaining on the floats. None of the 12 females was then less than 40 mm. in length and only one of them less than 50 mm. Approximately 27 per cent of the 41 males, on the other hand, measured less than 40 mm. and 54 per cent less than 50 mm. Those in the largest-sized group (70–79 mm.) were all females (Table I). Near the end of the spawning season (July 29) a collection of 70 individuals showed a sex ratio of 100 males to 23 females. Of those measuring less than 50 mm. in length there were 37 males but no females, while those of larger size comprised 15 males and 12 females. Only a single individual was still immature.

The correlation of sex and size is actually much closer than the tables indicate, for the figures given are based upon the length of the shell alone and not upon the actual size of the animal. Selection based on volume would undoubtedly give a still larger percentage of females at the end of the first year in the locality under consideration.

In other areas, however, the sex ratios at the end of the first year are quite different, as Needler (1932) has shown by comparing one-year-old oysters from Prince Edward Island, Long Island Sound, and Great South Bay, Long Island. She concluded that in the colder areas, with a shorter season of activity, relatively few individuals became sexually mature as males before their second year and none as females.

First-year Oysters from Quinnipiac River and New Haven Harbor

Collections were made monthly from September, 1931, to July, 1932, and a very large number of young oysters were examined. Nearly a hundred of these were cut in serial sections. Conditions were unfavorable for a large set in these areas during the summer of 1931 and there was a high mortality of the young oysters in exposed situations during the ensuing winter.

In November, when about four months of age, the shells of the largest individuals were from 20 to 27 mm. in length, but the majority measured only 5 to 15 mm. The gonads of the smaller individuals were beginning to branch out beneath the body walls, while those of the larger animals had already reached the primary bisexual phase, with differentiated ovocytes and spermatocytes (Figs. 2–6). A few spermatids were also present in some individuals.

The rate of growth and the accompanying sexual changes through the ensuing months and until the breeding season in July are summarized in Table IV. The table shows that the first individuals to become recognizably differentiated sexually were all males, and none of these could be identified with certainty until April or May. During June there was a relatively rapid growth in size, accompanied by an increasing number of sexually differentiated males among the larger individuals. But the correlation of size with sexual differentiation is not without exceptions, as Tables I and IV will show, although the sexual conditions may be definitely controlled by nutrition. Spermatogenesis or ovogenesis may be inaugurated when nutritive conditions are favorable but growth may then be checked by the encroachment of other individuals, while gametogenesis continues. The result may be a dwarfed but sexually functional animal.

TABLE IV

Correlation of Age, Size and Sex, Quinnipiac River and New Haven Harbor

Length	Nov.	Dec.	Mar.	May			July				
	Im.	Im.	Im.	Im.	M.	F.	Im.	M.	H.	F.	Total
mm.											
5– 9......	Many	Many	Many	Many	0	0	0	0	0	0	0
10–14......	Many	Many	Many	Many	0	0	2	0	0	0	2
15–19......	Some	Many	Many	Many	0	0	6	11	0	0	17
20–24......	Few	Some	Some	Some	Few	0	9	41	0	0	50
25–29......	Few	Few	Few	Few	Few	0	0	104	1	2	107
30–34......	0	0	0	Few	Few	0	0	116	1	3	120
35–39......	0	0	0	0	0	0	0	82	2	2	86
40–44......	0	0	0	0	0	0	0	26	0	3	29
45–49......	0	0	0	0	0	0	0	7	0	1	8
50–54......	0	0	0	0	0	0	0	2	0	2	4
Total....	—	—	—	—	Few	0	17	389	4	13	423

An examination of 423 yearlings taken at random at the height of the breeding season in July indicated that about 96 per cent of the survivors from the set of the previous year had become sexually mature. Most of these were males filled with spermatocytes and ripe spermatozoa (Table IV). The other 4 per cent still retained the primary intersexual gonads and indicated that sexual maturity had been postponed until their second year. All of these immature individuals, as would be expected, showed evidence of unfavorable nutritive conditions as indicated by their dwarfed size.

The predominantly protandric nature of the group is shown by the fact that 389, or nearly 96 per cent of the 406 sexually mature individuals examined were more or less fully ripe males, while only 13, or 3.2 per cent, were females. Four, or 1 per cent, were functional hermaphrodites with large masses of both ova and spermatozoa. The ratio of males to females is thus 100 : 3.3.

While the number of females is too small to be statistically dependable, it will be observed that their mean length is somewhat greater than that of the males, but less conspicuously so than in the much larger group from West Sayville (Table I). If the volumes had been measured, instead of the lengths, the females would have shown a still greater comparative size.

Comparison of Tables I and IV will show that the proportion of individuals which mature as females during their first year at West Sayville and in New Haven Harbor is about ten times as great in the former locality as in the latter. The size differences of the two populations of yearlings show that not only is there a correlation between rate of growth and sex as concerns individuals but that the proportion of females is several times higher in the locality where the conditions for growth are the more favorable or the growing season longer. There may be some question as to whether the evidence is sufficient to justify the conclusion that metabolic conditions at the time of sexual differentiation actually determine the direction taken by the primary intersexual gonad in its transformation into the functional organ of the first sexual phase. But until further experimental evidence is available it seems to be the most reasonable hypothesis suggested.

First Year Oysters from Near Woods Hole, Massachusetts

Young oysters from small natural beds in the vicinity of Great Harbor, from the shores of the neighboring islands, and from Onset were examined in the summers of 1931 and 1932. In all of these areas growth is slow during the first year and the shell seldom reaches a length exceeding 30 to 35 mm. The more usual length is 6 to 20 mm.

An examination of 389 yearling oysters from these localities showed that 373, or nearly 96 per cent, were still immature or the sex undeterminable, 9 were males, 3 were hermaphrodites and 4 were females. At the age of two years, when most of the young oysters in that region become sexually mature, the ratio of males to females is about one hundred to fifty-five, with approximately two per cent true hermaphrodites. At this age the average size of the females considerably exceeds that of the males, as was also the case in the other areas during the first breeding season.

Comparison with Other Species

The primary bisexuality of *O. virginica* and the development of the definitive gonads, including later sex reversals, are in many respects similar to the series of sexual phases which characterize such strictly hermaphroditic and larviparous species as *O. edulis* (Orton, 1926–27) and *O. lurida* (Coe, 1931, 1932). In both the hermaphroditic and seasonally dioecious types the more rapid proliferation of spermatogonia as compared with the ovocytes soon gives the early gonad its predominantly male characteristics. In the former type, however, all individuals are thought to become functional males before assuming the female phase, while in *O. virginica* from 3 to 30 per cent of the sexually mature young individuals at different localities show only an abortive male phase, the primary gonad developing into an ovary without completed spermatogenesis.

In each type residual cells of both sex lines remain after the first spawning, providing a cellular mechanism which leads in some of the hermaphroditic forms to a series of alternating sexual phases, while in *O. virginica* the sexual changes appear to be more or less facultative, for it is known that in at least some individuals the same sexual phase may be retained for several years. This is presumably true of the great majority of adult oysters under a stable environment.

Evidences of protandry and sex change have been reported in other species of oviparous oysters. Of a large number of very young *O. cucullata* examined by Roughley (1928) all except about 5 per cent were males and he suspected that these exceptions might previously have spawned as males. He found nine functional hermaphrodites. These were thought to represent stages in the transformation of male to female, but it seems more probable that the sexual conditions in that species are not very different from those here described for *O. virginica*, and that the change of sexuality in both species takes place in the interval between two breeding seasons. Among 3000 large adult oysters from thirty different localities he found a sex ratio of 270 females to 100 males.

In *O. angulata* also hermaphroditism occurs occasionally. Amemiya (1925) found two such individuals among 14 males and 59 females. But it is not known whether that species, which is closely allied to *O. virginica*, experiences similar sex changes.

A most interesting type of sex change has been recently reported by Amemiya (1929) for the Japanese oyster (*O. gigas*), previously considered dioecious. In one summer a small hole was made in the shell of each of several hundred oysters and the sex thereby determined. The sexes were then placed in separate cages and returned to the sea. A year later it was found that oysters of both sexes were present in each cage. It was concluded that about 25 per cent of the females and 60 per cent of the males had changed their sex during the winter. If these conclusions prove to be well founded, Amemiya's hypothesis, that the sex of any individual of that species is determined each winter independently of its previous sexual conditions, will add a new phase to the many variants of sexuality in animals.

Since there is such a wide diversity in the abundance and size of the ovocytes in the sexually mature young males of *O. virginica*, it is pertinent to inquire whether there may not be two genetically distinct types of these males. The samples studied indicate, as shown in Table II, that at West Sayville about 48 per cent of the entire one-year group or more than 70 per cent of the sexually mature yearlings function as males, while in New Haven Harbor there are fully thirty males to one female at their first breeding season (Table IV). It is conceivable that those males with but few and very small ovocytes are genetically "true males" while those with more numerous and larger ovocytes may represent the protandric males. Perhaps it is only the latter that later undergo sex reversal, as Orton (1928) has suggested for *Patella*. It would probably be unwise to speculate further in this connection until more complete evidence is available. The appearance of the primary gonads, however, and the changes which they subsequently undergo, suggest that sexuality in this species may rest upon a basis somewhat comparable with that which Witschi (1932) has found in certain races of frogs.

Summary

1. Examination of the developing gonads of young oysters from various localities at frequent intervals during the first two years of life shows that a primary bisexual gonad is formed in each individual within a few months after setting.

2. The activities of the gonad depend upon the temperature of the water and apparently other conditions of nutrition, a much larger proportion of the animals becoming sexually mature during the first year in warmer than in cooler localities.

3. The primary gonad contains the antecedent cells of both sexes, with ovocytes upon the walls of the follicles and spermatocytes intermingled and bordering the lumens.

4. The protandric nature of the primary gonad frequently becomes manifest by the rapid proliferation of the spermatogonia and the formation of primary spermatocytes; the latter soon pass through the synaptic phases and lead to the production of secondary spermatocytes

and spermatids at the age of a few months. But no functional spermatozoa have been observed until the following spring in the areas investigated. The species is not strictly protandric, however, for 3 to 30 per cent of the sexually mature yearlings are females, the ovaries of which have developed directly from the primary gonads without the completion of a preliminary functional male phase.

5. The definitive sexual gland is a transformation of the primary gonad by the proliferation of spermatogonia and the disintegration of many of the ovocytes to form the spermary or, less frequently, the growth of ovocytes, accompanied by the disintegration of spermatocytes and such spermatids as may be present, to form an ovary. But the intersexual character is usually retained to at least some extent in both types of gonads.

6. The proportion of male and female cells in the mature gonad is highly variable, a few large ovocytes being frequently found in some parts of otherwise typical spermaries, while in the ovary some follicles may retain characteristic male cells. True hermaphroditism was found in 1 to 4 per cent of the sexually mature oysters at the end of their first year. Apparently normal development follows self-fertilization.

7. In the warmer of the two principal localities investigated about 70 to 80 per cent of the oysters which became sexually mature during their first year were males. In the cooler locality the proportion of males exceeded 95 per cent.

8. Most of the relatively small number of females are among the largest of their age group, indicating a close correlation between sex and size. This may imply that the female is metabolically the more active sex or that she requires better nutritive conditions in order to mature, or that sex in this species is so labile that the nutritive conditions of the individual at the critical period of sex differentiation determine which of the alternative types of cells in the primary bisexual gonad shall predominate. Alternative genetical explanations are discussed.

9. After the animal has spawned as male or female the gonad may still retain its bisexual character; the sexual phase during the following year may again depend largely upon nutritive conditions.

10. The primary bisexuality of this species and the cellular mechanism for sex reversal here reported are interpreted with reference to related species of the genus in which hermaphroditism and alternating sexual phases have been retained.

LITERATURE CITED

Amemiya, I., 1925. Hermaphroditism in the Portuguese Oyster. *Nature*, **116**: 608.

Amemiya, I., 1929. On the Sex-change in the Japanese Common Oyster, Ostrea gigas Thunberg. *Proc. Imper. Acad. Tokyo*, **5**: 284.

Burkenroad, M. D., 1931. Sex in the Louisiana Oyster, Ostrea virginica. *Science*, **74**: 71.

Coe, W. R., 1931. Sexual Rhythm in the California Oyster (Ostrea lurida). *Science*, **74**: 247.

Coe, W. R., 1932. Development of the Gonads and the Sequence of the Sexual Phases in the California Oyster (Ostrea lurida). *Bull. Scripps Inst. Oceanog., Tech. Ser.*, **3**: 119.

Galtsoff, Paul S., 1932. Spawning Reactions of Three Species of Oysters. *Jour. Wash. Acad. Sci.*, **22**: 65.

Hoek, P. P. C., 1883–84. De Voortplantingsorganen van de Oester: Les organes de la genération de l'huître. *Tijdschr. Nied. Dierkund. Vereeniging*, Supplement **1**: 113–253.

Needler, Alfreda Berkeley, 1932. Sex Reversal in Ostrea virginica. *Cont. Can. Biol. and Fish.*, **7**: 285.

Orton, J. H., 1926–27. Observations and Experiments on Sex-change in the European Oyster (O. edulis). *Jour. Mar. Biol. Assoc.*, **14**: 967.

Orton, J. H., 1928. Observations on Patella vulgata. Part I. Sex Phenomena, Breeding and Shell Growth. *Jour. Mar. Biol. Assoc.*, **15**: 851.

Prytherch, Herbert F., 1924. Experiments in the Artificial Propagation of Oysters. *Bur. Fish. Doc.* No. **961**: 1–14. (App. xi, *Rept. U. S. Comm. Fish. for 1923.*)

Roughley, T. C., 1928. The Dominant Species of Ostrea. *Nature*, **122**: 476.

Spärck, R., 1925. Studies on the Biology of the Oyster (Ostrea edulis) in the Limfjord, with Special Reference to the Influence of Temperature on the Sex Change. *Rept. Dan. Biol. Sta.*, **30**: 1.

Stafford, Jos., 1913. The Canadian Oyster, its Development, Environment and Culture. *Comm. of Conservation, Canada:* 1–159.

Witschi, Emil, 1932. Physiology of Embryonic Sex Differentiation. *Am. Nat.*, **66**: 108.